

## **Delta-Selective Glycopeptides Related to Enkephalin Produce Profound Analgesia with Reduced Side Effects in Mice**

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### ***ANALGESICS BASED ON ENDOGENOUS NEUROPEPTIDES***

*The use of endogenous neuropeptides such as enkephalins and endorphins as drugs has remained an elusive goal since the 1970's. The principle reason for this is that peptides generally do not cross the blood-brain barrier, and are quickly degraded in the blood stream prior to delivery to opiate receptors in the brain. Animal research with glycosylated enkephalins and endorphins (dynorphins) indicates that potent analgesia is possible after intravenous or sub-cutaneous injection. Glycopeptides derived from delta-selective opioid agonists have 2-3X the potency of morphine, and lack many of the side effects associated with mu-agonists such as morphine. Morphine is still used on the battlefield for combat casualty care, and it is anticipated that further development of the glycopeptide analgesics will result in superior analgesics with greatly reduced side effects. Recent developments in this area are reported.*

### **1.0 INTRODUCTION**

There is no question that the American Civil War was a medical learning experience for the doctors involved in it. The American Civil War provided the setting for the first genuinely effective care of combat casualties with the introduction of field hospitals on or near the battlefield, and early treatment of casualties. (Figure 1) The pharmacopoeia of the day was not extensive by today's standards, but among the most effective agents were ether and chloroform anaesthetics used during amputations and other procedures, and morphine, used for the treatment of pre- and post-operative pain.<sup>1</sup> In the South, the scarcity and expense of imported drugs forced the Confederate Army to establish several medical laboratories to manufacture drugs for military use.<sup>2</sup> Empirical testing in military hospitals helped determine the clinical value of indigenous remedies. During this war morphine, both in its pure form and in various impure preparations of opium, gained its first widespread use on the battlefield, and in hospitals far removed from the field of battle. While there have been many advancements and refinements in combat casualty care in the intervening 130 years, morphine and its congeners are still used extensively, with many of the same unwanted side effects that were noted by the physicians of the 1860's. Chief among these unwanted side effects were respiratory depression and lowered blood pressure. It will never be known for certain, but it is very likely that opiates given to Stonewall Jackson in the course of his "diligent care" contributed to his death 8 days after the successful amputation of his left arm. It has recently been concluded that hemorrhagic shock and pneumonia, both possible sequelae of opiate administration, contributed to the death of this Confederate general, and consequently dealt a serious blow to the Confederate cause.<sup>3</sup> The problems of opiate induced respiratory depression are followed closely by the problems associated with tolerance and physical addiction. So widespread was the problem of opiate addiction of former soldiers after the war that it was given the term "veteran's disease."

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**Figure 1: Mathew Brady recorded the medical treatment of Union casualties in the American Civil War. Amputations were performed in the field (left), either with or without the benefit of chloroform or ether as an anaesthetic. If the wounded were lucky enough to make it to a hospital (right), pain was generally treated with various preparations of opium or morphine. At the time of the Civil War, and afterwards, opiate addiction was referred to as “the veteran’s disease.”**

## **2.0 ENDOGENOUS OPIOID PEPTIDES**

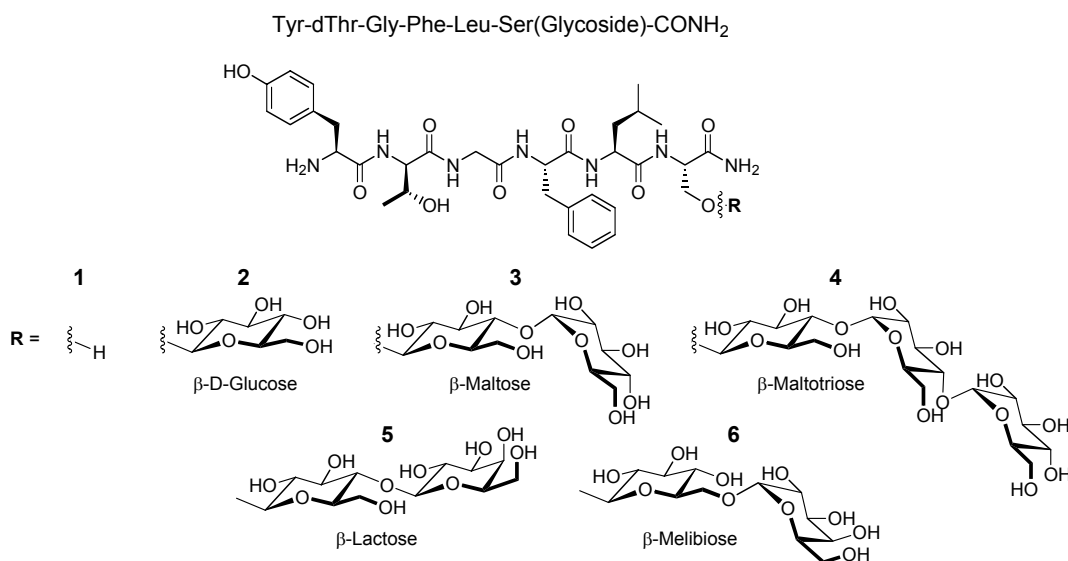
Long before the discovery of the opioid peptides, it was suspected that mammals produced an endogenous substance with morphine-like effects. Eventually, with the aid of immunocytochemistry, these substances were discovered, and eventually isolated and chemically characterized. Three major classes exist: the relatively large dynorphins and endorphins (sometimes collectively referred to as endorphins), and the much smaller enkephalins (methionine enkephalin and leucine enkephalin). All of these peptides are enzymatic hydrolysis products of much larger precursor proteins that have a wide variety of neuropeptides embedded within their sequences. The enzymatic cleavage of these precursor peptides into the neurotransmitters and neuromodulators that are secreted by neurons allows for many pathways for regulation, and is a complex issue that will not be discussed here.<sup>4</sup>

## **3.0 ENHANCED STABILITY AND BBB TRANSPORT OF GLYCOPEPTIDES**

With the discovery of the endogenous opiate peptides in the 1970’s, and the recognition of their high selectivity and potency, it was initially anticipated that a new pharmacopoeia based on met-enkephalin, leu-enkephalin, or  $\beta$ -endorphin would emerge. Since these peptide opiates are degraded to pharmacologically inert amino acids, whereas morphine and similar alkaloidal pharmaceuticals produce a cascade of biologically active metabolites, it was logically (and correctly) assumed that peptide analgesics would possess a limited side effect profile. Problems associated with the physicochemical features of peptides, including their metabolic liability have been largely solved in the intervening years with the introduction of un-natural and/or D-amino acids, and by covalent modifications of the peptide backbone. Unfortunately, the pharmacodynamic behaviour of most peptides is still poor, and the blood-brain barrier (BBB) remains as a significant and largely unsolved deterrent to the effective delivery of peptide-based central analgesia. The BBB is not only a physical barrier represented by the tight junctions of the cells of the brain microcapillaries, but is also an enzymatic barrier caused by a broad spectrum of proteolytic enzymes and specific peptidases.

A significant advance was made in the transport of enkephalins was reported in 1994, when it was noted that glycosylated enkephalins penetrate the BBB to produce centrally mediated analgesia in mice after *i.v.* injection. A series of glycopeptides were synthesized<sup>5</sup> with varying types of O-linked glycosides attached to

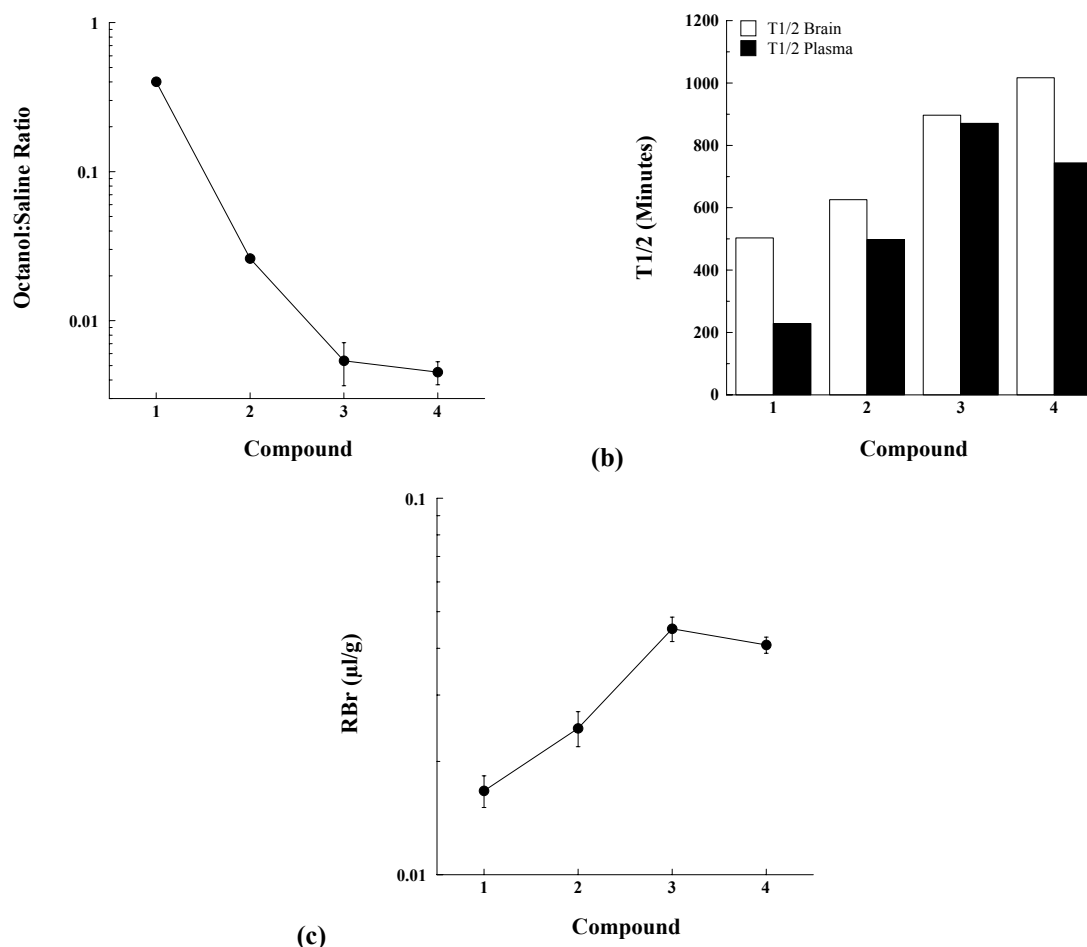
Ser<sup>6</sup> of a potent  $\delta$ -selective sequence first studied by Roques<sup>6</sup> (**Figure 2**). O-Linked glycosylation of the relatively lipophilic Leu-enkephalin C-terminal amide YdGFS\*-CONH<sub>2</sub> led to enhanced surfactant properties<sup>7</sup> of the molecule, which in turn led to increased interaction with membranes and membrane mimics.<sup>8</sup> Although these relatively short glycosylated neuropeptides had no defined conformation in aqueous solution (*e.g.* they existed as random coils), in the presence of sodium dodecyl sulphate (SDS) micelles or other membrane mimics they adopted a very restricted and well-defined set of conformations, as indicated by circular dichroism (CD) and <sup>1</sup>H-NMR analysis.<sup>9</sup>



**Figure 2: Glycosylated Enkephalin Analogues.** Glycosyl hexapeptides were synthesized using solid-phase Fmoc chemistry. The Fmoc serine glycosides were incorporated as the peracetates, and synthesized using methods developed in the Polt group.

YdGFLS*-CONH <sub>2</sub> Glycoside	Glucoside Moiety	$\delta$ Binding (nM)	$\mu$ Binding (nM)	MVD IC <sub>50</sub> (nM)	GPI IC <sub>50</sub> (nM)
<b>1</b> (peptide control)	—	2.1	7.5	2.7	25
<b>2</b> (glucomorphin)	β-D-Glc	2.4	7.6	1.6	34
<b>3</b> (maltomorphin)	α-D-Glc-(1→4)-β-D-Glc	9.9	30.8	1.7	52.6
<b>4</b> (maltotrimorphin)	[α-D-Glc-(1→4)] <sub>2</sub> -β-D-Glc	3.8	15	7.7	71.7
<b>5</b> (lactomorphin)	β-D-Gal-(1→4)-β-D-Glc	17.3	40	5.72	34.8
<b>6</b> (biomorphin)	α-D-Gal-(1→6)-β-D-Glc	5.6	36.6	6.06	43.8

**Table 1: *In Vitro* Binding Activity and Functional Assays for Glycosylated DTLES.** IC<sub>50</sub>'s for  $\delta$ - and  $\mu$ -opioid binding were determined using displacement of <sup>3</sup>H-labeled radioligands from rat brain homogenates. Functional assays were performed using electrically stimulated mouse *vas deferens* and *guinea pig illium*.

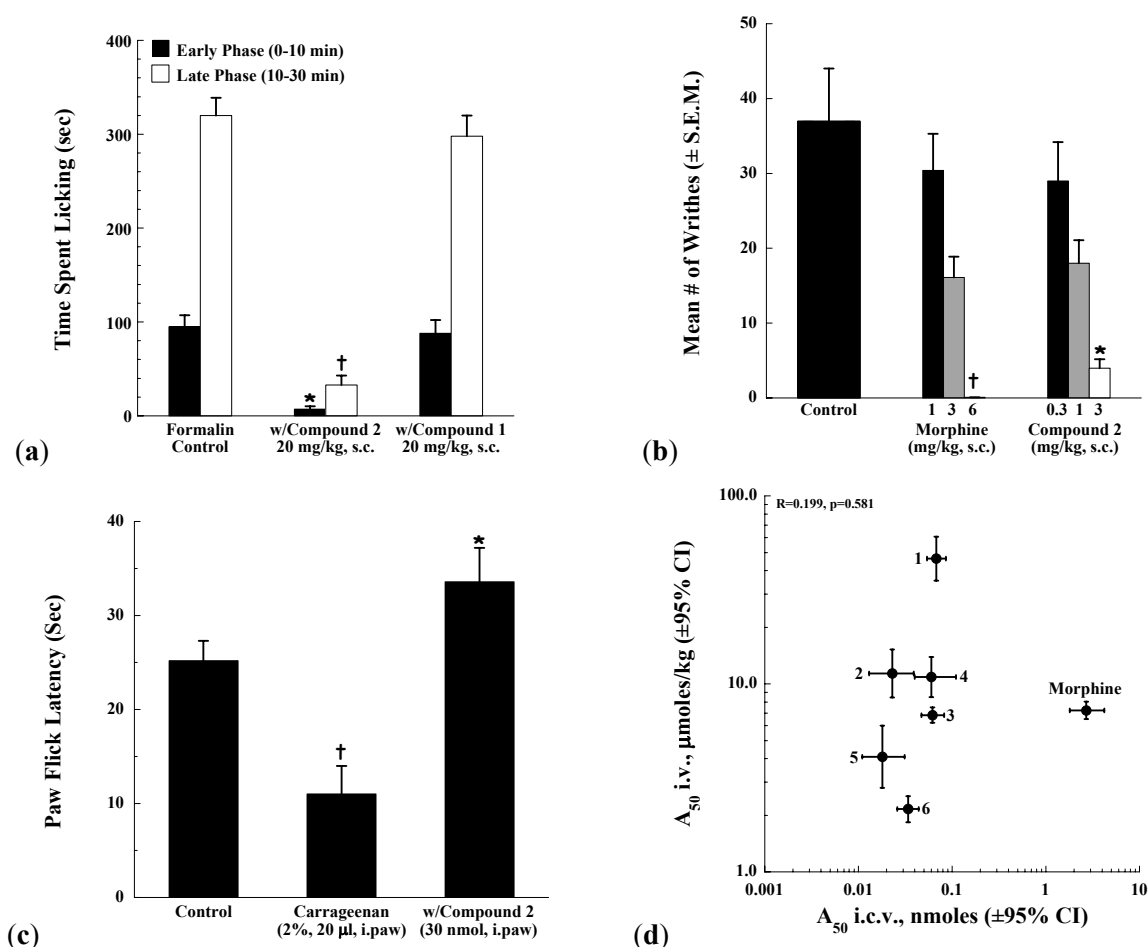


**Figure 3: Glycopeptide Stability and Transport.** (a) Octanol-saline distribution for the unglycosylated peptide 1 and glycopeptides 2, 3, and 4. The addition of 1, 2, or 3 glucose units to the opioid peptide message significantly decreases lipophilicity. (b) The *in vitro* stabilities of the peptide and glycopeptides were measured in mouse brain and serum. Increased glycosylation led to significant increases in stability in both brain and serum. Brain stability increased with each additional glucose. However, in the serum, the stability of the trisaccharide was lower than that of the disaccharide. (c) Brain delivery of the peptides measured by *in situ* perfusion studies. Addition of glucose to the peptide significantly increased uptake. Uptake to the brain was improved further for the disaccharide, giving the maximal delivery. The trisaccharide produced no further increase in BBB penetration.

Classical pharmacological theories of BBB transport suggest that peptides are not lipophilic enough to diffuse into the brain.<sup>10</sup> Glycosylation decreases lipophilicity even further. Despite this, greatly increased transport rates in rat brain have been observed for the glycosylated enkephalins (**Figure 3**). Previous studies with the glucoside **2** indicated that the increased transport was due to a saturable mechanism, thus further ruling out simple diffusion. Reversible interaction of the glycopeptides with the membrane is believed to promote transport through the brain capillaries by transcytosis.<sup>11</sup> Several other possible modes of transport (simple diffusion and receptor-mediated processes) have been ruled out.<sup>12</sup> Maximum transport rates (and maximum biological effects) are observed when the optimum degree of glycosylation is achieved. For this peptide, the disaccharide produces both the optimal transport and stability *in vivo*. In general, glycosylation leads to

enhanced stability of the peptide “message” in both serum and brain. The identity of the individual sugars does, however, contribute to the overall biological effect, which is a product of both BBB transport rates and the stability of the peptide in serum, as well as metabolism and excretion by the liver and kidneys.

## 4.0 ANALGESIC EFFECTS OF GLYCOSYLATED ENKEPHALINS

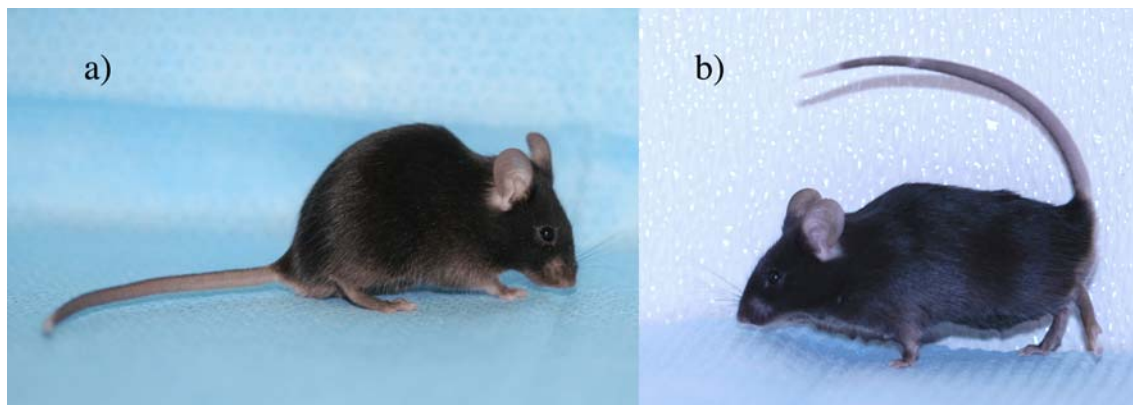


**Figure 4: Antinociception *in vivo*.** The glycosylated enkephalins showed strong analgesic activity in tests of antinociception after peripheral administration, which are more clinically relevant than the tail flick assay. (a) Mouse formalin paw test, glycopeptide 2, s.c. (b) Mouse abdominal constriction test, glycopeptide 2, s.c. (c) Mouse paw inflammation test with carrageenan, glycopeptide 2, *i. paw*. Injection of glycopeptide 2 into the contralateral injection had no antinociceptive effects. (d) Antinociceptive effects (mouse tail flick) of glycosylated enkephalins ( $A_{50}$  values) after *i.c.v.* injection (X axis), and after *i.v.* injection (Y axis). Morphine has been included as a reference point, but has been excluded from the correlation values, shown on the upper left part of the diagram. The observed analgesia after *i.v.* injection correlates most strongly with glycopeptide stability (Fig. 3b), and brain transport values (Fig 3c), rather than the *i.c.v.* potency.

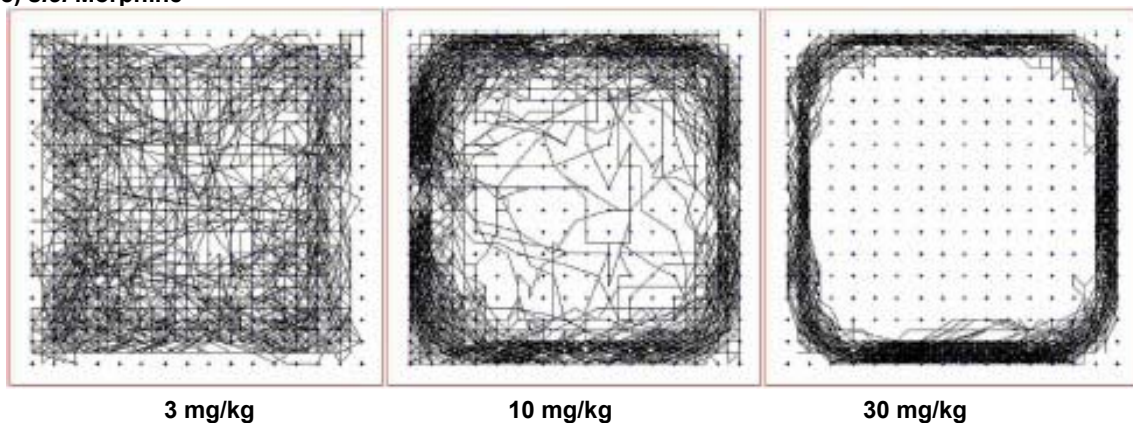
The extent of antinociception was shown to be comparable to, or even superior to the effects of morphine in mice after *i.c.v.* and *i.v.* administration<sup>13</sup> using the warm water tail flick assay.<sup>14</sup> The representative glycopeptides all produced full agonist effects in these assays with the potencies exceeding that of morphine



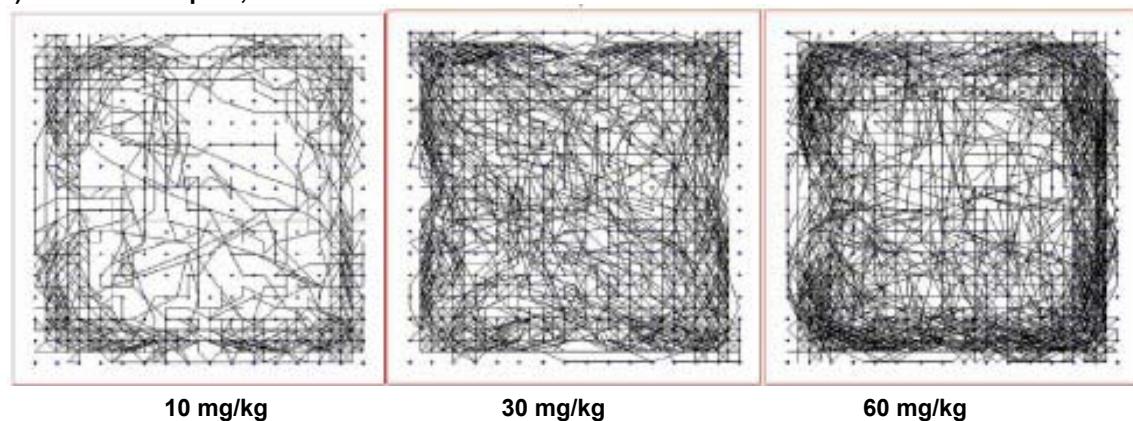
on a  $\mu\text{mol/kg}$  basis in some cases. (**Figure 4**) Additional analgesic assays involving visceral, chemical and inflammatory pain states were also used to gauge the effectiveness of **2** and **5** after *i.v.* and *s.c.* injection.



**c) s.c. Morphine**



**d) s.c. Glucomorphin, 2**



**Figure 5: Non-analgesic effects of opioids on mice. Both mice have received equi-analgesic ( $A_{90}$ ) doses of drug. (a) and (b) Glycopeptide-based analgesia did not induce Straub tail. (c) Morphine-induced analgesia induced large increases in locomotor activity, stereotypic circling, compared to equi-analgesic doses of glycopeptide 2 (d).**

Two well-known effects of morphine in rodents are increases in locomotor activity<sup>15,16</sup> with stereotypic patterns of movement,<sup>17</sup> and increases in muscular rigidity, including Straub tail.<sup>18</sup> Unlike morphine and other  $\mu$ -selective opioids, at equivalent *s.c.* A<sub>90</sub> antinociceptive doses, or even supramaximal doses, the glycopeptide analgesics produced minimal increases in locomotor activity, and did not produce Straub tail (**Figure 5**). These results were confirmed in two different strains of out-bred mice.

## 5.0 MECHANISM OF TRANSPORT

Evidence obtained from *in vivo*<sup>19,20</sup> as well as *in vitro* experiments<sup>9</sup> with the glycopeptides are consistent with an endocytotic mechanism of transport. Receptor mediated transport and diffusive mechanisms have been ruled out, and further work strongly suggests that adsorption to the endothelial membrane of the brain capillaries is required for BBB transport. While the drug must adsorb strongly to the membrane in order to undergo endocytosis or transcytosis, this must also be a reversible adsorption, otherwise the drug will bind tightly to the first membrane it sees, resulting in poorer transport. This concept is demonstrated clearly with the amphipathic  $\alpha$ -helices, **14**, **15**, and **16**. (Table 3)

Our work began with glycosylated enkephalins that were designed to have potent  $\delta$ -agonist activity, but still have appreciable  $\mu$ -agonist activity. While it is possible to produce some analgesic effects through the  $\delta$ -receptor alone, previous work has shown that  $\mu$ -agonists are much more effective in this regard. It was hoped that mixed  $\delta/\mu$ -agonists would show reduced side effects, relative to  $\mu$ -selective agonists, *e.g.* morphine. Other researchers have proposed  $\mu$ -agonist/ $\delta$ -antagonists as drug candidates for analgesia with reduced side-effects.<sup>21</sup> An important aspect that is not fully understood is the role that “address” segments play in determining receptor selectivity.

Helices are the most commonly occurring secondary structural elements in globular proteins, accounting for one-third of all the residues.<sup>22</sup> Linus Pauling first proposed the  $\alpha$ -helix as an important motif of secondary structure in proteins in 1948,<sup>23</sup> interestingly, without any experimental evidence.<sup>24,25</sup> Segrest first theorized the *amphipathic* (a.k.a. amphiphilic) helix to be an important structural motif of integral membrane proteins in 1974.<sup>26</sup> It is estimated that over 50% of all  $\alpha$ -helices in nature are amphipathic.<sup>27</sup> These proteins are unique in that they possess hydrophobic and hydrophilic parts either by primary structure (highly hydrophilic N-terminus and hydrophobic C-terminus) or by secondary structure, with polar residues pointing one to face and the non-polar residues on the opposite face. This allows them to “float” in a cell membrane, exposing the hydrophilic side to the aqueous exterior of the cell and the hydrophobic side to the lipophilic membrane.<sup>28,29</sup> This peptide-membrane interaction is believed to be important for two reasons. First, the amphipathic nature of the helix can help guide a drug or hormone to its specific receptor by narrowing the receptor search from a 3-dimensional search to one in 2-dimensions. Surface-assisted “reduction-of-dimensionality” calculations, performed by Polya in 1921, were examined by Max Delbrück in which he quantitatively demonstrated the viability of this theory.<sup>30</sup> Assuming that no other forces are at work (*e.g.* convection), and that the membrane is fluid, the probability of a substrate finding its corresponding receptor is much better in 2-dimensions (*e.g.* a cell surface) than in 3 (*e.g.* in solution)—almost 100% when the search is reduced to 2-dimensions.

Second, membrane insertion may allow the portion that interacts with the receptor (pharmacophore or “message”) to be fixed in a specific geometry. By restricting mobility in the membrane near the binding site, the amphipathic  $\alpha$ -helix can dramatically alter the peptide-receptor interaction.<sup>31</sup> In addition, membrane insertion can also induce a specific conformation in the ligand, different from its solution conformation. It seems clear that the bioactive conformation of a peptide is the membrane-bound conformation, and that membrane insertion is actually the first step in receptor activation.



The endogenous neuropeptide  $\beta$ -endorphin is a 31-residue naturally occurring opioid peptide. The first 5 residues of  $\beta$ -endorphin are identical to Met-Enkephalin. It has been shown that the  $\alpha$ -helical structure of C-terminal region of  $\beta$ -endorphin play a role in the receptor binding and opiate activities, and resistance to proteolysis.<sup>32</sup> Kaiser<sup>33</sup> proposed that  $\beta$ -endorphin consists of the [Met<sup>5</sup>]-enkephalin peptide sequence at the N-terminus, a hydrophilic linker region from residues 6—12, and an amphiphilic helical region between the residues Pro<sup>13</sup> and Gly<sup>30</sup>, which were assumed to be “helix breakers.” This hypothesis has been supported by the conformational analysis of a number of  $\beta$ -endorphin mimics with artificial C-terminal helical regions with amphipathic character.<sup>34,35,36</sup> All of the analogues were  $\alpha$ -helical by CD measurements, as the monomer or oligomers, and showed strong opioid agonism *in vitro* when compared to natural  $\beta$ -endorphin. These studies clearly suggest that amphipathicity of the entire peptide is more important than the identity of specific amino acids present in the helical C-terminus.<sup>37</sup> This has been further supported by the work by Kyle,<sup>38</sup> who synthesized several potent peptide analogues containing the  $\alpha$ -helix-promoting residues  $\alpha$ -aminoisobutyric acid (Aib) and N-methyl alanine (MeAla) near the C-terminal region of nociceptin, the natural ligand for the recently identified opioid receptor-like 1 receptor (ORL-1). According to Schwyzner,<sup>39</sup> the N-terminal “message” is steered toward certain receptors and away from others by the C-terminal “address” segment, which interacts with the membrane to orient the message with respect to the receptor.

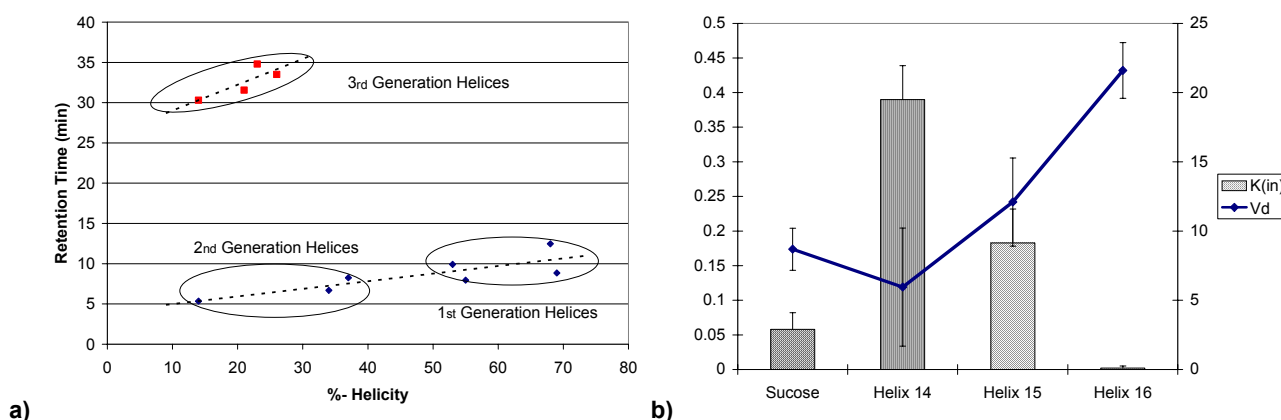
Dynorphin A (1-17) is an endogenous opioid heptadecapeptide which binds preferentially to the  $\kappa$  opioid receptor.<sup>40</sup> Dynorphin consists of a N-terminal message identical to Leu-enkephalin, and an address sequence that imparts selectivity for  $\kappa$  receptors.<sup>41</sup> Dynorphin A is believed to adopt an extended and/or random coil structure as determined by various spectroscopic measurements.<sup>42,43,44,45,46</sup> In the presence of DPC micelles Dynorphin A is believed to contain a less ordered N-terminus, a well defined  $\alpha$ -helix segment spanning between Phe<sup>4</sup> and Pro<sup>10</sup> or Lys<sup>11</sup> and a  $\beta$ -turn from Trp<sup>14</sup> to Gln<sup>17</sup>.<sup>47</sup> Based on NMR results, the authors concluded that both the  $\alpha$ -helix and the C-terminal  $\beta$ -turn may be a consequence of dynorphin’s interaction with the micelle, and may be important structural features of the full-length peptide when bound to the cell membrane *in vivo*. The  $\alpha$ -helix could have multiple roles in positioning the amphipathic helix for interaction with the receptor, as amphipathic helices have many roles at interface.

Helix Glucoside	Glycopeptide Sequence	Retention Time (RP-HPLC)	% Helicity (CD)	<i>i.c.v.</i> Analgesia IC <sub>50</sub> (picoMol)
7	YtGFLGELAS*KWFNALE	8.85 min	69%	insoluble
8	YtGFLGELAS*KWFNALES*	7.95 “	55%	270
9	YtGFLGELAS*KWFNALES*F	9.91 “	53%	insoluble
10	YtGFLGELAS*KWFNALES*FW	12.48 “	68%	insoluble
11	YtGFLGLLKS*FAES*WS*NF	6.69 “	34%	~ 30
12	YtGFLGKS*FAELWS*NFLS*	5.35 “	14%	~30
13	YtGFLGLLKS*FWES*WS*NF	8.25 “	37%	~30

**Table 2: Glycosylated Endorphin Analogues.**

Helix Glucoside	Glycopeptide Sequence (3 <sup>rd</sup> Generation)	Retention Time (RP-HPLC)	Per Cent Helicity (CD)	MVD IC <sub>50</sub> (nM)	GPI IC <sub>50</sub> (nM)
<b>14</b>	YtGFL( <b>P</b> )NLBEKALKS*L-CONH <sub>2</sub>	31.57	21	34.5	63.1
<b>15</b>	YtGFL( <b>βA</b> )NLBEKALKS*L-CONH <sub>2</sub>	33.50	26	23.0	354
<b>16</b>	YtGFL( <b>GG</b> )NLBEKALKS*L-CONH <sub>2</sub>	30.30	14	18.8	196
—	Morphine	—	—	258	54.7

**Table 3: Glycosylated Endorphin Analogues.**

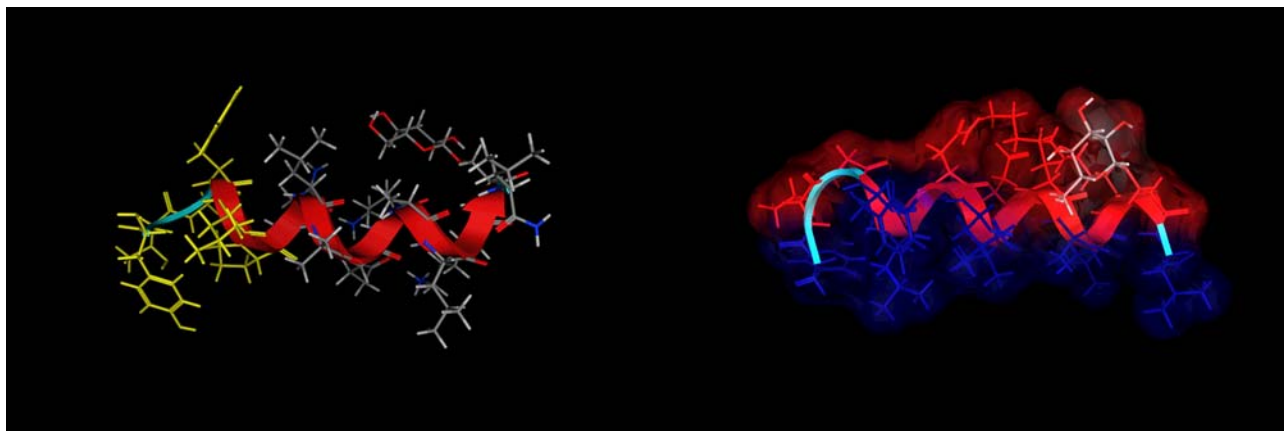


**Figure 6: (a) Plot of Retention Time vs Degree of Helicity. (b) Mouse BBB Transport Data.**

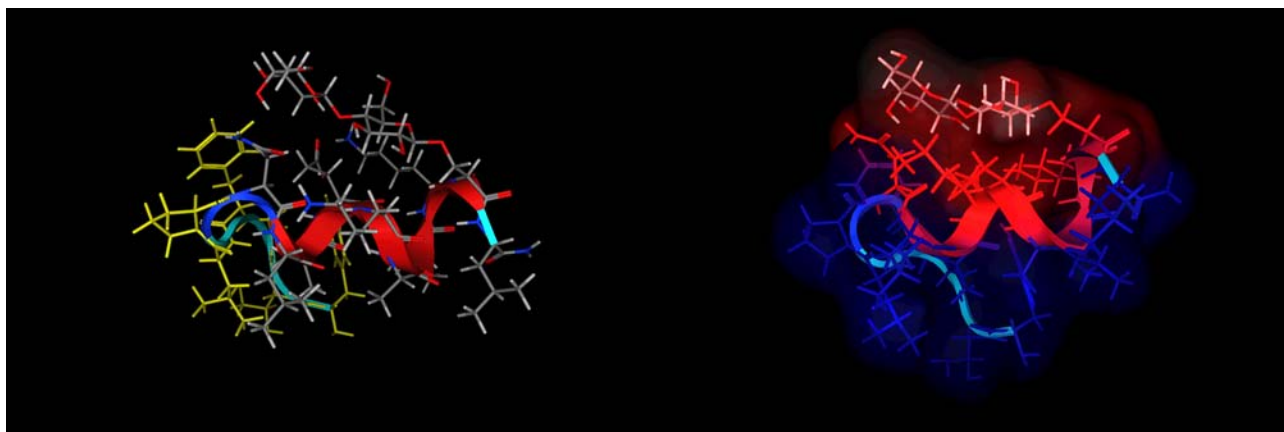
The first- and second-generation endorphins were also based on the  $\delta$ -selective YdGFL~ opioid message. Formed by simple truncation, the first generation helices, 7—10, were designed to probe the minimum length for helix formation. Essentially, we overshot the target, and all of these compounds were extremely helical, but they were not water soluble enough to work with, with the exception of helix 8. This compound possessed appreciable antinociceptive activity, however.<sup>48</sup> All of these compounds were quite soluble in the presence of SDS micelles. Since these compounds are so stable in their helical form, they probably form aggregates, and fall out of solution in the absence of the detergent. The second generation helices, 11—13, were designed to be less lipophilic, and consequently were more water soluble, and showed much less helicity in the presence of micelles.<sup>49</sup>

The third-generation helical endorphin-based glycopeptides, 14—16, used the same  $\delta$ -selective peptide DTLET first studied by Roques, and showed much superior properties, both in the chemistry lab and in the mouse. Using *in situ* methods in the mouse, not rat studies as before, Egleton was able to measure BBB transport rates independently of analgesia, and Bilsky has been able to demonstrate the analgesic effects of these larger glycopeptides using *i.c.v.* tail flick results in the mouse.<sup>50</sup> Initial studies with these glycohexadecapeptides indicated that BBB transport rates were determined by the amphipathic nature of the glycopeptides,<sup>51</sup> rather than the lipophilicity of the compound, *per se*,<sup>52</sup> and that they actually show BBB transport rates that are similar to, or better than the shorter enkephalin analogues.

These endorphin analogues have the same N-terminal YdGFL~ opioid message contained in the enkephalin analogues **1**—**6**, and the same C-terminal amide address sequence ~NLBEKALKS\*L-CONH<sub>2</sub>, where B is the helix-stablizing  $\alpha$ -aminoisobutyric acid (Aib) residue, and S\* is the serine glucoside residue. The “linker region,” which is intended to “break” the helix, and prevent propagation of the helical address into the opioid message, is different in the three glycopeptides: **14** => proline, **15** =>  $\beta$ -alanine, and **16** => glycylglycine.



**Figure 7: Lipid Bound Helix.** One structure of glycopeptide **14** in the presence of micelles, as determined by NOE-constrained molecular dynamics calculations. The message segment is labelled in yellow, and the helix indicated with the overlaid ribbon. The structure on the right has the hydrophobic (blue) and hydrophilic (red) surfaces labelled. The structures were rendered with the MOE<sup>®</sup> software package.



**Figure 8: Lipid Bound Helix-Bend.** One structure of glycopeptide **17** in the presence of micelles, as determined by NOE-constrained molecular dynamics calculations. The message segment is labelled in yellow, and the helix indicated with the overlaid ribbon. The structure on the right has the hydrophobic (blue) and hydrophilic (red) surfaces labelled. The structures were rendered with the MOE<sup>®</sup> software package.

While the data presented in Figure 6 is interesting, and perhaps even compelling, it is also clear that one cannot only use the degree of helicity to predict amphipathicity. NMR evidence, in conjunction with Monte Carlo calculations (NOE constraints not discussed here) shows that the glycopeptides bind to micelles, and

adopt a very restricted set of conformations. For the helices **14**, **15**, **16**, and the disaccharide **17** (not pictured in Table 3, but is the top-most data point in Figure 6a) we see two membrane bound conformational ensembles, one that is very helical, (*e.g.* Figure 7) and one that has a helix-bend motif (*e.g.* Figure 8), but is none-the-less very amphipathic. The peptide sequence for **17** is the same as the sequence for **14**, but the compounds differ in that **14** is glycosylated with the  $\beta$ -D-glucoside, and **17** is glycosylated with the disaccharide  $\beta$ -lactose. These two compound both show the same conformations in their micelle-bound ensembles based on NMR, and similar helicities based on CD, but slightly different population densities.

While it there is still much to be learned about the details of both the transport and binding processes of the amphipathic glycopeptides, an important principle has emerged concerning transport. It seems clear that one must have a glycopeptide that essentially has two states: 1) A state defined by one or more membrane-bound conformations that permit or promote endocytosis. 2) A state defined by a water-soluble, or random coil state that permits “membrane hopping.” The key to efficient transport is to balance these two states so that the compound is neither retained in the membrane, or held in solution so that it cannot undergo adsorptive endocytosis. It may also be true that aggregation of glycopeptides on a membrane surface may actually initiate and promote endocytosis.

## 6.0 CONCLUSIONS

Based on the results obtained so far, it would seem that further pre-clinical studies are warranted to test the viability of the glycosyl enkephalin analogues (*e.g.* compounds **2**, **5** or **6**) as a replacement for morphine on the battlefield. Anecdotal studies in mice suggest that these compounds possess an extremely low level of toxicity, even at super-analgesic doses. The notion that one could administer a large sub-cutaneous dose of a non-toxic glycopeptide that would have prolonged analgesic effects without respiratory depression or the risk of overdose is particularly appealing. Further research needs to be completed in order to quantify the effects of the glycosylated  $\delta$ -agonists on respiration and blood pressure, particularly in hypovolemic animals to gauge the propensity of these compounds to induce hemorrhagic shock. Complete absorption, metabolism and excretion studies (ADME) need to be completed, and oral bioavailability needs to be explored. The fact that the glycosylation strategy seems to be effective with the much larger endorphin analogues (*e.g.* compounds **14** and **15**) suggest that this approach may have general applicability to BBB transport of non-analgesic (or even non-opioid) neuropeptides, which could lead to novel treatments for anxiety, stress-related disorders and depression.

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